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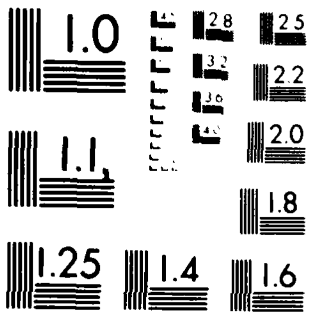

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Annual Report

Saint Louis Encephalitis

Temperature-Sensitive Mutants

Thomas A. Brawner, Ph.D.

September 1979

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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University of Missouri

School of Medicine

Columbia, Missouri 65212

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ABSTRACT

Conditional lethal temperature-sensitive (ts) mutants are defined by their inability to replicate at a nonpermissive temperature. Generally, this temperature is above 37°C. Temperature-sensitive mutants have often been used to examine the molecular mechanisms of viral replication. At the present time, the events involved in St. Louis encephalitis virus RNA and protein synthesis are poorly understood. This may be due, in part, to a lack of a population of stable ts mutants. As a first step in the development of such a population, a heat resistant viral clone was developed by multiple cycles of incubation at 60°C. The resulting clone was mutagenized by direct treatment witn N-methyl-N'nitro-N-nitrosoguanidine (NTG) or incorporation of the base analogues 5-azacytidine (5-Aza C) or 5-fluorouracil (5-FU) into replicating viral RNA. Treatment of virions with NTG resulted in the examination of 1273 plaques. However, no ts mutants were observed. Virus from cells exposed to 5-FU without actinomycin D pretreatment was examined. Two ts-mutants were identified after examination of 507 plaques. Cells pretreated with actinomycin D and infected were exposed to 5-FU. Four of 868 plaques examined yielded ts mutants. One hundred thirty six plaques isolated from pools of virus grown in the presence of 5-Aza C were examined. No ts mutants were isolated. However, virus infected cells pretreated with actinomycin D yielded 14 ts mutants when 625 plaques were examined. Reversion frequencies ranged from 1.16×10^{-4} to $< 8.1 \times 10^{-8}$ for ts mutants induced with 5-FU and from 7.5×10^{-2} to $< 1.39 \times 10^{-8}$ for ts induced mutants with 5-Aza C.

The RNA phenotype of several ts mutants have been examined. The results indicate that three mutants have a reduced ability to incorporate RNA precursors at the nonpermissive temperature (40°C) and three incorporate near normal levels.

Several mutants have been used in pairwise crosses to determine complementation

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SUMMARY

Temperature-sensitive mutants have been examined to determine their ~~(1)~~ ability to incorporate ^{3}H-uridine into viral specified RNA and ~~(2)~~ complementation patterns. Results of RNA precursor incorporation experiments suggest that mutants may be separated on their ability to synthesize RNA at the nonpermissive temperature.

Several mutants have been examined to determine their complementation groups. No complementation has been observed with the very few mutants examined.

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I. Statement of the problem:

The 1977 report from the Center for Disease Control indicated that of the 2,599 cases of encephalitis associated with viral illness 2,113 or 81% were the result of togaviral infections. SLE virus was confirmed as the cause of 86% with 142 resulting in death. These statistics emphasize the fact that SLE has been considered to be the prime cause of viral encephalitis in the United States.

Presently, no vaccine is available to protect against St. Louis encephalitis (SLE) or many other alpha or flavivirus infections.

The systematic production and evaluation of strains with potential to protect a given population is essential.

II. Background

Most live virus vaccines are composed of an attenuated population of viruses. The process of attenuation usually involved continuous passage in an animal or cell culture. This process results in the uncontrolled production of viruses with a reduced virulence. More recently, an examination of viruses with an altered ability to replicate at increased temperatures has resulted in the observations that they, in many cases, are less pathogenic (Ghendon, et. al., 1973, Wagner, 1974, Brown et al., 1975).

A careful examination of ts mutants has suggested that these may, in fact, be good candidates for attenuated, live virus vaccines. The results of Ghendon (1973) indicate that a large number of polio virus ts mutants producing a pathologic change in infected monkeys were assayed for virus production. Results of assaying the isolated virus at permissive and nonpermissive temperatures indicates that a great deal of reversion is seen, with the isolated virus reflecting the temperature profile of the parental "wild type" virus.

However, the results presented by Brown, et al., (1975) indicate that when the ts mutants is carefully selected the virus seen in tissues are not the result of reversion but are the product of limited virus replication at body temperature.

The results of Wagner (1974), Brown, et al., (1975) and Harrison, et al 1977) ts mutants used as live virus vaccines will confer protective immunity. One author (Wagner, 1974) suggests that the immunity observed in animals infected with vesicular stomatitis virus RNA$^-$, ts mutants is the result of residual protein synthesis (Wunner and Pringle, 1972). Brown et al, (1975) tested a number of Eastern encephalitis virus ts mutants and reported the ts mutants, mainly RNA$^-$ mutants, would provide protection against challenge by the wild type virus. These experiments suggest that ts mutants may be a valuable source of viruses for vaccines.

III. Approach to the Problem:

At the present time, there is no vaccine to protect against an infection by SLE nor many of the other togaviral diseases. An effort needs to be made to identify the characteristics of effective vaccine strains of SLE. This information could result in not only selecting a vaccine strain of SLE but in selecting candidate vaccine strains of other togaviruses.

The nature of the viral vaccine used is of great importance. Information from studies with Poliovirus has shown that the use of live attenuated virus vaccines result in longer lasting, more effective immunity to challenge by the wild type virulent, virus. This proposal is an effort to systematically develop and select attenuated viral strains with the characteristics of good vaccines. These characteristics are (1) few or no symptoms upon infection (2) induction of long lasting protective immunity against wild type virus (3) and no reversion to the wild type. Temperature-sensitive mutants may provide effective protection against challenge by wild type strains.

IV. Results

The temperature-sensitive mutants listed in the original grant proposal and in Table 1 constitute the working stocks of virus available for this study. Several of the mutants were examined to determine their ability to incorporate RNA precursors at the nonpermissive temperature. These results are shown in Table 2. Three of the mutants, 100-01, 100-02 and 100-R4 incorporated only about 40% of the label incorporated by the parental. The remaining mutants tested incorporated near normal levels.

Several of the mutants were used in mixed and paired single infections to determine complementation patterns. These results are shown in Table 3. It is generally considered that the complementation frequency, a comparison of the mixed infection titer to the sum of individual infections, should be greater than two if complementation has occurred. As can be seen from these results, none of the levels are higher than one.

The data gathered during the past five months have provided a start toward complementation analysis and RNA phenotyping of our population of mutants. The negative results of the complementation assay are not disturbing. Only a few mutants have been examined and the techniques may need to be refined.

Examination of the RNA phenotype has been encouraging and will be continued and extended to many more mutants.

V. Conclusions

The data presented is a small portion of the anticipated total. With this in mind, it is not proper to draw many conclusions. These results do suggest that RNA incorporation experiments to determine phenotype may yield interesting results. However, it is too early to determine the out come of complementation experiments.

With the above information in mind, the following recommendations are put forth:

1. Complementation experiments be continued and expanded to include more mutants, 2. RNA phenotype experiments be continued and 3. present pools of ts mutants be screened to look for more possible mutants.

TABLE 1

Temperature --Sensitive mutants of St.Louis encephalitis virus induced with 5-fluorouracil

Mutant Designation	P.F.U./ml		Reversion Frequency
	$30^{0}C$	$40^{0}C$	
D6-200-F	2.3×10^{8}	< 5	$< 2.2 \times 10^{-8}$
1000-C	2.5×10^{8}	2.9×10^{4}	1.2×10^{-4}
1000-I	2.7×10^{8}	< 5	$< 1.9 \times 10^{-8}$
25-e	6.2×10^{7}	< 5	$< 8.1 \times 10^{-8}$
200 f	1.0×10^{7}	9.5×10^{1}	9.5×10^{-6}
500 b	3.5×10^{7}	5.5×10^{3}	1.6×10^{-4}
2000 f	1.1×10^{7}	< 5	$< 4.5 \times 10^{-7}$

TABLE I (continued)

Temperature-Sensitive mutants of St. Louis encephalitis virus induced with 5-azacytidine

Mutant Designation	P.F.U./ml 30°C	P.F.U./ml 40°C	Reversion Frequency
25-A	2.0×10^6	<5	$<2.5 \times 10^{-6}$
100-B	1.0×10^4	<5	$<5.0 \times 10^{-4}$
100-O	2.5×10^6	<5	$<2.0 \times 10^{-6}$
100-S	6.0×10^6	<5	$<8.3 \times 10^{-7}$
100-24	4.5×10^5	<5	$<1.1 \times 10^{-5}$
100-26	3.2×10^5	<5	$<1.6 \times 10^{-5}$
100-32	1.6×10^7	<5	$<3.1 \times 10^{-7}$
100-35	1.6×10^7	1.2×10^1	7.5×10^{-7}
100-38	1.5×10^6	<5	$<3.3 \times 10^{-6}$
100-41	3.5×10^6	3.5×10^3	1.0×10^{-3}
100-2	3.3×10^7	<5	$<1.5 \times 10^{-7}$
100-8	1.7×10^7	<5	$<2.9 \times 10^{-7}$
100-b	1.2×10^6	9.0×10^4	7.5×10^{-2}
100-r_4	1.1×10^6	<5	$<4.6 \times 10^{-6}$
100-25	6.8×10^7	2.8×10^4	4.1×10^{-4}
100-O_1	1.4×10^8	8.2×10^2	5.7×10^{-6}
100-O_2	3.6×10^8	<5	$<1.4 \times 10^{-8}$
100-r_1	3.5×10^7	<5	$<1.4 \times 10^{-7}$
100-s_1	2.8×10^6	<5	$<1.8 \times 10^{-6}$

TABLE 2

Incorporation of RNA precursors into replicating viral RNA

Mutant Number	Mutagen	RNA synthesis (% of control)
SPI-T_4	-(control)	100
200f	5-Fu	100
100-35	5-Aza C	95
100-36	5-Aza C	75
100-$_{01}$	5-Aza C	40
100-$_{02}$	5-Aza C	36
100$_{-4}$	5-Aza C	38

TABLE 3

Complementation analysis of temperature-sensitive mutants

Mutant Designation

	25e	100-01	$D_2$1000i	D_2D_1 1000g
25e	-	.127	1.042	.608
100-01		-	.610	ND*
$D_2$1000i			-	-
$D_2D_1$100og				

ND=Not determined

Literature Cited

Brown, H.R. Vosdingh and E. Zebovitz (1975)
Attenuation and immunogenicity of _ts_ mutants of Eastern Encephalitis virus for mice. J. Gen. Virol. _27_:111-116.

Ghendon, Y.Z., A.T. Marchenko, S.G. Markushin, D. Ghenoria, A.V. Mikhejeva, and E.E. Rozina (1973).
Correlation between _ts_ phenotype and pathogenicity of some animal viruses. Arch gesamte Virus. _42_:154-159.

Harrison, V.R., K.H. Eckels, J.W. Sagartz and P.K. Russell (1977).
Virulence and immunogenicity of a temperature-sensitive Dengue-2 virus in lower primates. Infect. Immun. _18_: 151-156

Wagner, R.R. (1974)
Pathogenicity and immunogenicity for mice of temperature-sensitive mutants of Vesicular Stomatitis virus. Infection and Immunity _10_:309-315.

Wunner, W.H. and C.R. Pringle (1972)
Protein synthesis in BHK-21 cells infected with Vesicular Stomatitis virus I. _ts_ mutants of the Indiana serotype. Virology _48_:104-111.

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